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Familial Ring (19) Chromosome Mosaicism: Case Report and Review

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Ring (19) chromosomal mosaicism has been identified in a 14-month-old girl referred for cytogenetic evaluation due to microcephaly and developmental delay with autistic-like mannerisms. An analysis of her peripheral blood lymphocytes showed a 46,XX,r(19) cell line in 119/121 of cells examined. Of the two remaining cells, one had a normal female chromosome complement and the other showed loss of one of the chromosome 19 homologs. Further analysis by fluorescence in situ hybridization using an all human telomere probe showed the presence of a single hybridization signal on the r(19) chromosome. Subsequent cytogenetic characterization of cells derived from the patient's phenotypically normal mother also demonstrated the presence of a ring 19 chromosome in 4/100 cells. The remaining cells had a normal female chromosome complement. These findings represent the first reported case of familial ring 19 mosaicism. The cytogenetic and clinical findings in these two individuals are discussed in relation to six previously reported cases of de novo ring chromosome 19 mosaicism.

KEY WORDS: mosaicism, microcephaly, chromosome 19, ring chromosome

INTRODUCTION

Rings derived from each human chromosome have been reported; yet, as a class of chromosome abnormalities, they represent relatively rare events [Wyandt, 1988]. This is particularly true of the smaller acrocentric and metacentric chromosomes including chromosome 19. To date, six individuals with ring chromosome 19 (r(19)) have been described [Uchida and Lin, 1972; Jacobs et al., 1978; Sybert et al., 1988; Gillessen-Kaesbach and Ngo, 1990; Yung et al., 1990; Sawyer et al., 1993]. In each case, cytogenetic studies of lymphocyte and/or fibroblast cell cultures documented mosaicism with both normal cells and cells in which one chromosome 19 was replaced by a ring chromosome. Clinical evaluations of those six individuals showed that two were phenotypically normal and four were clinically affected with manifestations ranging from minor anomalies to profound mental retardation. As with other autosomal ring carriers, the lack of a distinct genotype-phenotype correlation is problematic, particularly in genetic counseling and clinical management. Three hypotheses put forth to account for the clinical variability among ring carriers include differences in the length of deleted DNA sequences resulting from ring formation, the variable proportions of normal to ring chromosome cell lines in different tissue types, and the instability common to ring chromosomes [reviewed in Kosztolányi et al., 1987].

We present the cytogenetic findings in a phenotypically normal woman and her clinically affected daughter, both of whom have ring 19 mosaicism. These individuals expand the clinical observations of de novo r(19) mosaicism and represent the first familial case of ring 19 chromosomal mosaicism.

MATERIALS AND METHODS

Clinical Report

CS was referred to the University of Utah Genetics Clinic at age 14 months for diagnostic evaluation of fetal alcohol syndrome suspected on the basis of her behavior. She was the term product of an uncomplicated pregnancy. Her mother, MR, was a 27-year-old G3P2 from Costa Rica who denied the use of illicit medications and drugs, including tobacco and alcohol, during pregnancy. She has had no medical problems and is of normal intelligence. CS was born at 38 weeks of gestation without complications; weight was 2,664 g (10th centile), length was 48.4 cm (50th centile), and OFC was 33 cm (10th centile). She was adopted at birth and her foster parents first became concerned about her development at age 6 to 8 months. CS rolled at 6 months

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and sat at 7 months, yet she did not crawl until 14 months. Even so, she persisted in not making much effort at locomotion. She had a one word vocabulary at 14 months at which time a developmental screening examination showed her to be at the 4 to 5 month stage in language, 9 month stage for gross motor, and 10 month stage for fine motor. She had stereotypic mannerisms that included hand opening and closing, and head banging. Physical examination at 14 months showed a well-nourished, microcephalic girl of normal appearance (Fig. 1). She was somewhat oblivious to others in the examination room and had poor eye contact. Her length was at the 60th centile, weight was at the 40th centile, and her OFC of 44 cm was below the 5th centile. She had a central occipital hair whorl, bitemporal narrowing, palpebral fissure length of 2.5 cm (75th centile), and bilateral overlap of the 2nd and 4th toes on the 3rd toe. She has a normal brain MRI, negative urine metabolic screen, normal hearing, and a mildly abnormal electroencephalogram suggestive of static encephalopathy and thought to contribute to periodic staring episodes. A chromosome study was indicated based on her developmental delay and microcephaly.

Cytogenetic Studies

Cytogenetic analysis was carried out on peripheral blood lymphocytes from CS and her biological mother MR. GTG-banding was performed following standard cytogenetic procedures [Seabright, 1971]. In each case, at least 100 metaphase cells were examined. Fluorescence in situ hybridization (FISH) using a digoxigenin-labeled all human telomere probe (Oncor, Gaithersburg, MD) was performed on metaphase chromosomes from peripheral blood lymphocytes from the proband; 25 metaphase cells were scored for the presence of signal on the r(19) chromosome.

RESULTS

The cytogenetic analyses demonstrated r(19) mosaicism in CS and her mother (Table I). Characterization of the ring chromosome in each individual showed no apparent visible loss of genetic material at a 550 banding resolution (Fig. 2). The frequency of the ring 19 observed in CS differed significantly from that seen in her phenotypically normal mother. Of 121 cells examined, 119 (98%) showed 46,XX,r(19), one cell showed loss of chromosome 19 and one cell was 46,XX. In contrast, an analysis of 100 cells from her mother documented 96 cells with a normal female chromosome complement and four cells with 46,XX,r(19).

FISH analysis of the r(19) chromosome in the proposita, using an all human telomere probe, showed the presence of a single hybridization signal in each of 25 metaphase cells examined (Fig. 3).

With the exception of loss of the ring chromosome from one cell found in CS, the ring observed in both patients showed no evidence of instability generally associated with ring chromosomes including duplicated segments, double or multiple rings in a single cell, or di- or poly-centric ring chromosomes.

Fig. 1. CS at 14 months of age.

DISCUSSION

Ring chromosome 19 is a rare cytogenetic finding and has been described in only six previously reported cases [Uchida et al., 1972; Jacobs et al., 1978; Sybert et al., 1988; Yung et al., 1990; Gillessen-Kaesbach et al., 1990; Sawyer et al., 1993]. Of these patients, two were described as unaffected and four as affected. The frequency of the r(19) chromosome and the associated clinical phenotype of each patient are shown in Table I. Of the two cases of r(19) presented in this report, one individual was phenotypically normal with low grade mosaicism (4%) in peripheral blood. Her daughter, who has 98% r(19) cells in her peripheral blood, is microcephalic with developmental delay and autistic mannerisms. Together, the clinical descriptions of the eight cases demonstrate that there is not a common phenotype associated with r(19) mosaicism; three individuals were clinically normal, three had microcephaly without other malformations or minor anomalies, two were growth retarded, and three demonstrated developmental delay. As with other cases of chromosomal ring abnormalities, patients with r(19) do not demonstrate a clearly recognizable syndrome.

The lack of a distinct genotype-phenotype correlation among patients with r(19) is problematic, particularly in genetic counseling and in clinical management. A number of genetic mechanisms have been proposed to explain the clinical variability among these patients, and among ring chromosome carriers in general. One mechanism is breakage in both telomeric regions of the chromosome with loss of distal segments, followed by subsequent rejoining of "sticky ends." Although no detectable loss of chromosomal DNA was observed in any of the eight cases of r(19), chromosome 19 consists almost entirely of euchromatin and the detection of very small, submicroscopic deletions would be difficult to discern. Nevertheless, the phenotypic variability seen among the clinically affected patients with apparently identical rings may reflect differences in the location of the breakpoints, and in the length of small undetectable deletions resulting in a genetic imbalance. The

TABLE I. Frequency of r(19) in the Present Cases and in Six Previously Reported Patients

Reference	Tissue	46,XX or XY (%)	46,XX or XY,r(19) (%)	45,XX or XY,-19 (%)	Phenotype
Uchida et al., 1972	Peripheral blood	78	22		Normal
Jacobs et al., 1978	Peripheral blood	50	50		Profound MR, hearing loss, absent speech, club foot, nondysmorphic
Sybert et al., 1988	Peripheral blood (newborn)	90	10		Microcephaly, high forehead, high nasal bridge, low-set and posteriorly rotated ears, mild gross motor delay, redundant nuchal skin, pulmonic stenosis, hypoplastic right ventricle, coronary artery fistula
	Peripheral blood (2 months)	92	8		
	Fibroblasts, skin (2 months)	99	1		
	Fibroblasts, cardiac (6 months)	92	8		
	Peripheral blood (6 months)	84	16		
Yung et al., 1990	Peripheral blood (newborn)	79	19	2	SGA, microcephaly, growth retardation, syndactyly, digital hypoplasia
	Peripheral blood (3 years)	84	16		
	Fibroblasts, skin (3 years)	100			
Gillessen-Kaesbach et al., 1990	Amniotic fluid	30	70		Growth delay, prominent forehead, hypertelorism, prominent philtrum, retro- and micrognathia, cutis laxa, joint contractures
	Peripheral blood	20	80		
	Fibroblasts, tendon	30	70		
Sawyer et al., 1993	Peripheral blood		93	7	Normal
	Fibroblasts, skin	7	86	4; 3[a]	
Present case, proposita	Peripheral blood	1	98	1	Microcephaly, autistic behavior developmental delay
Present case, mother	Peripheral blood	96	4		Normal

[a] Other rearrangements of chromosome 19.

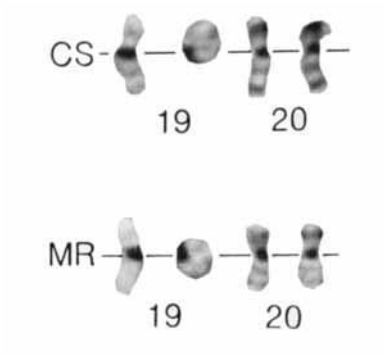

Fig. 2. Partial karyotypes showing the r(19) chromosome observed in CS and in her phenotypically normal mother MR.

second mechanism involves end-to-end fusion of palindromic DNA sequences at the telomeres without loss of euchromatin [Kosztolányi, 1987]. This type of ring formation could explain the clinically normal individuals reported by Uchida et al. [1972] and Sawyer et al. [1993] (Table I). However, FISH analysis of cells from our mildly affected proband (CS) with an all human telomere probe showed the presence of a single, distinct hybridization signal of the r(19) chromosome (Fig. 3).

The lack of a consistent phenotype for a smallest region of common deletion argues against a deletion as a cause of the abnormalities in clinically affected individuals. Furthermore, if a minimum region of deletion was involved in the formation of the ring, the clinical severity would be expected to correlate positively with

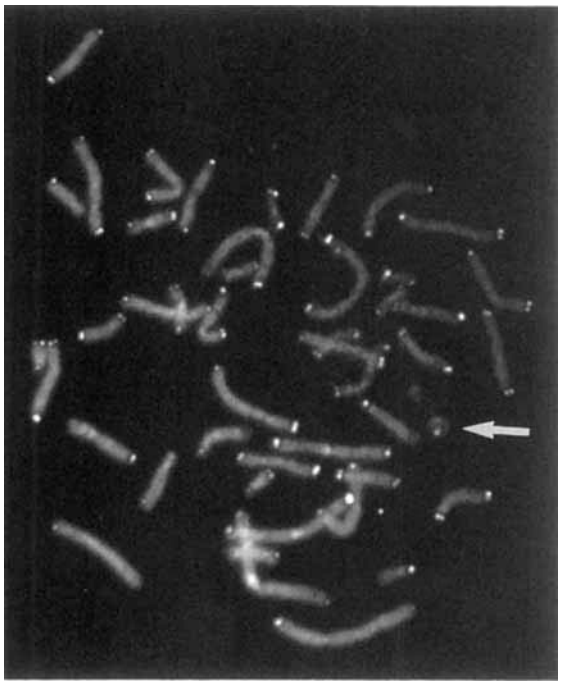

Fig. 3. Hybridization of an all human telomere probe to metaphase cells from the proband, CS. A single fluorescent signal was observed on the r(19) chromosome (arrow).

the percent of r(19) cells detected by karyotype analysis. However, a clear correlation does not exist among the four previously reported cases of de novo r(19) who are clinically affected. The most severely affected r(19) patient, described by Sybert et al. [1988] had the lowest percent of abnormal cells in both lymphocyte and fibroblast cell cultures. The index case in this report has the highest percent of r(19) cells, yet has no malformations and exhibits only microcephaly with non-specific developmental delay. The percent of r(19) cells in the other three affected patients does not correspond either to the degree of developmental delay or the level of somatic growth retardation. Thus, taken together the phenotypic variability among clinically affected individuals is not adequately explained either by a submicroscopic deletion of euchromatin in ring formation or by the degree of mosaicism.

A more perplexing cytogenetic finding is the segregation of ring chromosome mosaicism in families in which both clinically normal and abnormal individuals have been identified [Back et al., 1989; Kosztolányi et al., 1991]. A review of inherited ring chromosome case reports [Kosztolányi et al., 1991] showed nine families in which child and parent were both mosaics. In one third of the cases the phenotype of the offspring was very similar to that of the parent, in one third the offspring were more severely affected, and in the remaining families the offspring were less affected than their carrier parent. In the present study, both mother and daughter have an apparently identical r(19) chromosome by cytogenetic analysis, yet CS has developmental delay, microcephaly and minor anomalies while her mother apparently has a normal phenotype. Since no visible deletion is present on the r(19), and since telomeric sequences are present on the r(19) in the proposita, it is most likely that the ring chromosome found in the mother resulted from a telomeric fusion without deletion of euchromatin and she is therefore clinically unaffected. A small submicroscopic deletion may have occurred in the ring chromosome during maternal meiosis and an altered or deleted ring was subsequently passed onto CS via normal segregation thus giving rise to her abnormal phenotype. Alternatively, the substantial difference in the number of r(19) cells in mother (4/100) and daughter (119/121) may play a role in their differing clinical phenotypes. If the r(19) were to cause an abnormal cellular phenotype, then higher percentages of such cells could give rise to a clinically evident phenotype. One final hypothesis is that the ring chromosome observed in CS resulted from telomere association without genetic loss and therefore, the ring is a coincidental finding unrelated to her clinical manifestations which may be due to some other genetic or environmental mechanism.

Regardless, the unusual finding of inherited ring mosaicism in CS requires further explanation. Assuming that formation of the ring was a prezygotic event, the presence of normal cells would suggest that CS is a chimera. On the other hand, a postzygotic loss of the maternal ring chromosome followed by a duplication of the normal paternal chromosome 19 (uniparental disomy) would also explain the presence of a normal cell population. Alternatively, Back et al. [1989] have

proposed that a ring chromosome may be "predisposed" to terminal lesions or breaks and that chromosome instability is transmitted from parent to offspring. An inherited instability of that type would lead to de novo formation of the ring. It is also possible that the presence of only a single normal cell in peripheral blood from CS may represent an in vitro artifact resulting from reopening of the r(19) chromosome and CS may *not be mosaic* but rather has inherited a relatively stable r(19) from her mosaic r(19) mother. An analysis of additional cells from other tissue(s) would be required to rule out true mosaicism.

The two individuals described in this report represent the first reported case of familial r(19). Like other inherited ring chromosome reports, the phenotypic variability observed among relatives is difficult to reconcile but may be secondary either to differing amounts of DNA loss from chromosome 19 during ring formation or to the degree of mosaicism. It is equally possible that the rare cases of r(19) are serendipitous findings and the variable clinical findings in those individuals is unrelated to a r(19) karyotype. Further molecular characterization of the ring chromosome using probes and DNA markers that map to the distal regions of chromosome 19 may clarify any potential correlation between clinical phenotype and r(19) mosaicism.

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